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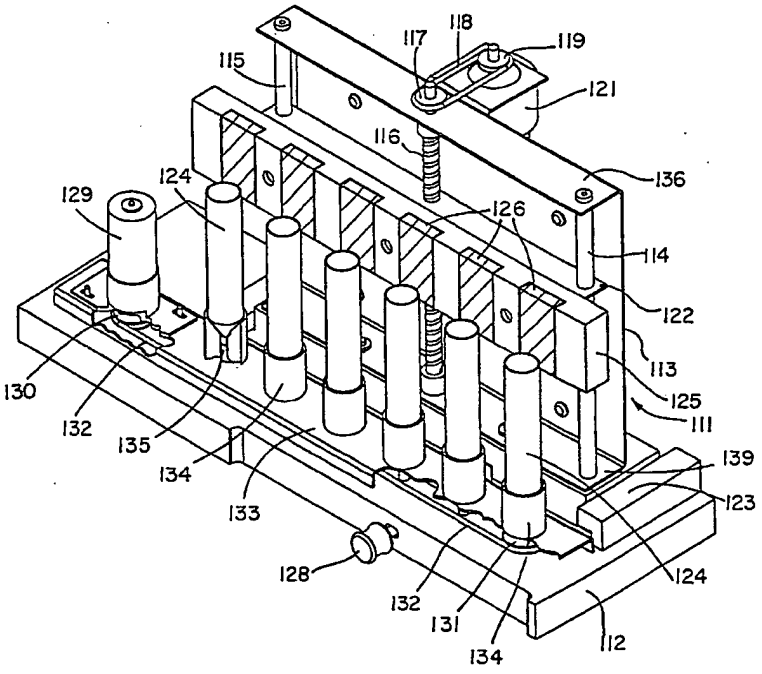

(57) Abstract: An apparatus and method for carrying out the affinity separation of a target substance from a liquid test medium by mixing magnetic particles having surface immobilized ligand or receptor within the test medium to promote an affinity binding reaction between the ligand and the target substance. The test medium with the magnetic particles in a suitable container is removably mounted in an apparatus that creates a magnetic field gradient in the test medium. This magnetic gradient is used to induce the magnetic particles to move, thereby effecting mixing. The mixing is achieved either by movement of a magnet relative to a stationary container or movement of the container relative to a stationary magnet. In either case, the magnetic particles experience a continuous angular position change with the magnet. Concurrently with the relative angular movement between the magnet and the magnetic particles, the magnet is also moved along the length of the container causing the magnetic field gradient to sweep the entire length of the container. After the desired time, sufficient for the affinity reaction to occur, movement of the magnetic gradient is ended, whereby the magnetic particles are immobilized on the inside wall of the container nearest to the magnetic source. The remaining test medium is removed while the magnetic particles are retained on the wall of the container. The test medium or the particles may then be subjected to further processing.

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APPARATUS AND METHOD FOR MIXING AND SEPARATION EMPLOYING MAGNETIC PARTICLES

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to an apparatus and a method for mixing and separation of magnetic Particles for the purpose of isolating substances of interest from a nonmagnetic liquid test medium.

2. Description of Related Art

Magnetic separation of biomolecules and cells based on magnetic particles and employing biospecific affinity reactions is advantageous in terms of selectivity, simplicity, and speed. The technique has proved to be quite useful in analytical and preparative biotechnology and is now being increasingly used for bioassays and isolation of target substances such as cells, proteins, nucleic acid sequences and the like.

As used herein, the term "receptor" refers to any substance or group of substances having biospecific binding affinity for a given liquid, to the substantial exclusion of other substances. Among the receptors susceptible to biospecific binding affinity reactions are antibodies (both monoclonal and polyclonol), antibody fragments, enzymes, nucleic acids, lectins and the like. The term "ligand" refers to substances such as antigens, haptens, and various cell associated structures having at least one characteristic determinant or epitope, which substances are capable of being biospecifically recognized by and bound to a receptor. The term "target substance" refers to either member of a biospecific binding affinity pair, i.e., a pair of substances or a substance and a structure exhibiting a mutual affinity of interaction, and includes such things as biological cells or cell components, biospecific ligands, and receptors.

Affinity separation refers to known process techniques where a target substance mixed with other substances in a liquid medium is bound to the surface of a solid phase by a biospecific affinity binding reaction. Substances, which lack the specific molecule or structure of the target substance, are not bound to the solid phase and can be removed to effect the

separation of the bound substance or vice versa. Small particles, particularly polymeric spherical particles as solid phase, have proved to be quite useful, as they can be conveniently coated with biomolecules, provide a very high surface area, and give reasonable reaction kinetics. Separations of the particles containing bound target substance (bound material) from the liquid medium (free material) may be accomplished by filtration or gravitational effects, e.g., settling, or by centrifugation.

Separation of bound/free fractions is greatly simplified by employing magnetizable particles which allows the particle bound substance to be separated by applying a magnetic field. Small magnetizable particles are well known in the art as their use in the separations involving immunological and other biospecific affinity reactions. Small magnetizable particles generally fall into two broad categories. The first category includes particles that are permanently magnetized, and the second comprises particles that become magnetic only when subjected to a magnetic field. The latter are referred to as paramagnetic or superparamagnetic particles and are usually preferred over the permanently magnetized particles.

For many applications, the surface of paramagnetic particles is coated with a suitable ligand or receptor, such as antibodies, lectins, oligonucleotides, or other bioreactive molecules, which can selectively bind a target substance in a mixture with other substances. Examples of small magnetic particles or beads are disclosed in U.S. Patent No. 4,230,685; U.S. Patent No. 4,554,088; and U.S. Patent No. 4,628,037. The use of paramagnetic particles is taught in publications, "Application of Magnetic Beads in Bioassays," by B. Haukanes and C. Kvam, Bio/Technology, 11:60-63 (1993); "Removal of Neuroblastoma Cells from Bone Marrow with Monoclonal Antibodies Conjugated to Magnetic Microspheres" by J. G. Treleaven et. al., Lancet, January 14, 1984, pages 70-73; "Depletion of T Lymphocytes from Human Bone Marrow," by F. Vartdal et. al., Transplantation, 43: 366-71 (1987); "Magnetic Monosized Polymer Particles for Fast and Specific Fractionation of Human Mononuclear Cells," by T. Lea et. al., Scandinavian Journal of Immunology, 22: 207-16 (1985); and "Advances in Biomagnetic Separations," (1994), M. Uhlen et. al. eds. Eaton Publishing Co., Natick, MA.

The magnetic separation process typically involves mixing the sample with paramagnetic particles in a liquid medium to bind the target substance by affinity reaction, and then separating the bound particle/target complex from the sample medium by applying a magnetic field. All magnetic particles except those particles that are colloidal, settle in time. The liquid medium, therefore, must be agitated to some degree to keep the particles suspended

for a sufficient period of time to allow the bioaffinity binding reaction to occur. Examples of known agitation methods include shaking, swirling, rocking, rotation, or similar manipulations of a partially filled container. In some cases the affinity bond between the target substance and the paramagnetic particles is relatively weak so as to be disrupted by strong turbulence in the liquid medium. In other cases biological target substances such as cells, cellular fractions, and enzyme complexes are extremely fragile and will likewise be disrupted or denatured by excess turbulence.

Excess turbulence is just one of several significant drawbacks and deficiencies of apparatus and methods used in the prior art for biomagnetic separations. The specified configuration of a magnetic separation apparatus used for separating particle-bound target complex from the liquid medium will depend on the nature and size of magnetic particles. Paramagnetic particles in the size range of 0.1 to 300 μm are readily removed by means of commercially-available magnetic separation devices. Examples of such magnetic separation devices are the Dynal MPC series of separators manufactured by Dynal, Inc., Lake Success, NY; and BioMag Separator series devices manufactured by PerSeptive Diagnostics, Cambridge, MA; and a magnetic separator rack described in U.S. Patent No. 4, 895,650. These devices employ permanent magnets located externally to a container holding a test medium and provide only for separation. Mixing of the paramagnetic particles in the test medium for affinity binding reaction must be done separately. For example, Dynal MPC series of separators requires a separate mixing apparatus, a Dynal Sample Mixer, for agitating the test media. The process must be actively monitored through various stages of mixing, washing, and separation, and requires significant intervention from the operator. Accordingly, the efficiency of these devices is necessarily limited by the skill and effectiveness of the operator.

U.S. Patent No. 4,910,148 describes a device and method for separating cancer cells from healthy cells. Immunoreactive paramagnetic particles and bone marrow cells are mixed by agitating the liquid medium on a rocking platform. Once the particles have bound to the cancer cells, they are separated from the liquid medium by magnets located externally on the platform. Although such mixing minimizes the liquid turbulence, it does not provide an efficient degree of contact between the particles and the target substance. Moreover, the utility of this device is limited to the separation of cells from relatively large sample volumes.

U.S. Patent No. 5,238,812 describes a complicated device for rapid mixing to enhance bioaffinity binding reactions employing a U-tube-like structure as mixer. The U-tube is rapidly,

rocked or rotated for 5 to 15 seconds to mix the magnetic particles in the test medium, and then a magnet is brought in close proximity to the bottom of the U-tube to separate the magnetic particles. As stated in the '812 patent, its utility is limited to treating very small volumes (< 1000 μl) of test medium.

U.S. Patent No. 5,336,760 describes a mixing and magnetic separation device comprising a chamber attached to a platform with one or more magnets located close to the container and an intricate mechanism of gears and motor to rotate the platform. Immuno-reactive paramagnetic particles are mixed in the test medium by first placing a stainless steel "keeper" between the chamber and the magnet to shield it from the magnetic field. Then the platform is rotated between vertical and horizontal positions. The particles in the test medium are mixed by end-over-end movement of the chamber. Following the mixing, the "keeper" is removed so that the magnetic particles are captured by the exposed magnetic field. Beside requiring a complicated mechanism, agitation of the liquid medium by end-over-end rotation does not mix relatively buoyant particles efficiently, and the liquid turbulence will tend to shear off or damage the target substance.

U.S. Patent No. 5,110,624, relates to a method of preparing magnetizable porous particles and describes a flow-through magnetically stabilized fluidized bed (MSFB) column to isolate proteins from cell lysate. The MSFB column is loosely packed with a bed of magnetizable particles and equipped with means of creating a stationary magnetic field that runs parallel to the flow of solution through the column. The particles are maintained in a magnetically stabilized fluidized bed by adjusting the rate of flow of the solution and the strength of the magnetic field. This is a complicated technique requiring precise adjustment of the flow rate and magnetic strength so that the combined effect of fluid velocity and magnetic attraction exactly counterbalances the effect of gravity on the particles. Moreover, the design of MSFB is not optimized for use with small test volumes, and cannot be made optimal for applications such as bioassays or cell separations.

International patent application WO 91/09308 published June 27, 1991 discloses a separating and resuspending process and apparatus. This application teaches that rotation of a magnet around the container containing paramagnetic particles induces the particles to remain as a compact aggregate (in close proximity to the magnet source) and roll over one another. The application teaches that this method fails to produce resuspension of the particles. The application discloses that the magnetic particles must be subjected to sequential magnetic fields

situated opposite each other in order to effect resuspension. The application describes a device comprising a chamber located between two electromagnets which are energized and de-energized to aggregate the magnetic particles alternately at the two magnets. The application teaches that alternately energizing and de-energizing the two electromagnets at a sufficiently rapid rate keeps the particles suspended in the center of the chamber. This method limits movement of the particles to a relatively small distance, significantly reducing the collision frequency between particles and the target substance, necessary for affinity binding which is a major reason for mixing the paramagnetic particles in the liquid medium. Moreover, a significant fraction of the particles, particularly particle-cell complexes may escape the magnetic field by gravitational settling to the bottom of chamber and will be lost during aspiration of the liquid medium following the aggregation step.

Japanese patent No. JP58193687 entitled Agitation And Separation Of Microscopic Material is directed to separation of microorganisms by mixing magnetized ultra-fine magnetic wire with microorganisms containing magnetic particles. The mixing is accomplished by a rotary magnetic field which also acts to separate the microorganisms after a mixing step. This patent is concerned with separation of microorganisms that contain internally ultra-fine magnetic particles. Such microorganisms are well known in the art, a particular example being magneto spirillium, a bacteria known to synthesize ultra fine magnetic particles. Such microorganisms would not and cannot be used as magnetic particles for mixing and separation of a target species as envisioned by the present invention. The Japanese patent's requirement for linearly-connected ultra-fine magnetic particles refers to a wire which is most likely used to create a high gradient magnetic field (HGMF) to collect or precipitate the magnetite-containing bacteria over the surface of these wires. Such a technique has no application to the process of affinity separation of a target substance from a liquid test medium as envisioned by the present invention since it relies on the magnetic properties of the microorganisms (the target substance itself) to effect a reaction.

The applicable known procedures have shortcomings, including the requirement for separate mechanically complex mixing mechanisms, as well as various process constraints and inefficiencies. The present invention provides devices and methods for magnetic mixing and separation which are of relatively simple construction and operation, which can be adapted to process large or small volumes of test liquid, and which can process multiple test samples simultaneously.

Additionally, the invention provides a single device for both mixing and separation and a method which maximizes the mixing efficiency of the paramagnetic particles in the liquid medium without causing detrimental liquid turbulence.

SUMMARY OF THE INVENTION

According to the present invention, the affinity separation of a target substance from a liquid test medium is carried out by mixing magnetic particles bearing surface immobilized ligands or receptors to promote specific affinity binding reaction between the magnetic particles and the target substance. The liquid test medium with the magnetic particles in a suitable container is removably mounted in the apparatus of the present invention. In one preferred embodiment, a single magnetic field gradient is created in the liquid test medium. This gradient induces the magnetic particles to move towards the inside Wall of the container nearest to the magnetic source. Relative movement between the magnetic source and the aggregating magnetic particles is started to mix the magnetic particles in the test medium and is continued for a sufficient time to ensure optimum binding of the target substance by affinity reaction. In addition, concurrently with the relative movement, the magnetic source may be moved from one end of the container to the other thereby effectively scanning along the length of the container by the magnetic field gradient. When the relative movement between the magnet and the magnetic particles is stopped, the magnetic particles are immobilized as a relatively compact aggregate on the inside wall of the container nearest to the magnetic source. The test medium may then be removed while the magnetic particles are retained on the wall of the container and may be subjected to further processing, as desired.

BRIEF DESCRIPTION OF THE DRAWINGS

The objects and features of the present invention, which are believed to be novel, are set forth with particularity in the appended claims. The present invention, both as to its organization and manner of operation, together with further objects and advantages, may best be understood by reference to the following description, taken in connection with the accompanying drawings, wherein:

Figures 1a, 1b, 1c, 1d, 1e and 1f schematically illustrate the steps of a method according to the invention for mixing and separation of a target substance employing magnetic particles.

Figure 2a shows a perspective top view of the use of two electromagnets placed at opposite sides of the container;

Figure 2b shows a perspective top view of the use of a ring of electromagnets surrounding the container;

Figure 3 shows a perspective view of the preferred embodiment of the invention which includes a row of magnets mounted on a vertically mobile assembly moveable by a linear drive mechanism and which can be positioned by a sliding mechanism at a desired distance from the corresponding rotatable containers which are rotated by a common mechanism.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The following description is provided to enable any person skilled in the- art to make and use the invention and sets forth the best modes contemplated by the inventor for carrying out his invention. Various modifications, however, will remain readily apparent to those skilled in the art, since the principles of the present invention are defined herein specifically to provide an apparatus and method for mixing and separating samples containing paramagnetic particles which maximize the mixing efficiency of the particles without causing significant liquid medium turbulence.

The invention permits rapid, efficient, and clean separation of a target substance from test media and is particularly useful in the affinity magnetic separations of organic, biochemical, or cellular components of interest from, for example, assay reaction mixtures, cell cultures, body fluids and the like. The invention includes a novel mixing system wherein the magnetic particles are mixed within a relatively motionless test liquid by magnetic means disposed external to the container holding the test liquid. The invention also includes an apparatus and method wherein magnetic particles while mixing and confused in a magnetic zone are concurrently linearly displaced to scan large volumes of test medium for affinity separation with a small concentration of magnetic particles. The invention provides an apparatus in which both the processes of mixing and separation are carried out by a common magnetic means disposed in a single apparatus, thereby making it simpler and more practical to use.

The apparatus of the invention comprises at least one container for holding a test medium, external magnetic means to generate a magnetic field gradient within the test medium,

and means for creating a magnetically-induced movement of the magnetic particles within the test medium. The apparatus of the invention may also include a linear motion mechanism to move the magnetic means for scanning a large volume of the liquid test medium. The container for performing the described mixing and separation is preferably of cylindrical configuration, made of a nonmagnetic material such as glass or plastic. Preferably, the container has at least one opening for receiving the test medium containing the magnetic particles.

The magnetic means may comprise one or more permanent or electromagnets disposed externally to the container for generating magnetic field gradients within the liquid test medium. In a preferred embodiment, the magnet is a permanent magnet of a rare earth alloy such as anisotropic sintered materials composed of neodymium-iron-boron or samarium-cobalt. The magnet is disposed external to the container so as to define a magnetic field gradient cavity in a desired cross-section of the test medium. The term cavity is employed because the magnetic field gradient acts to confine or concentrate the magnetic particles much as were enclosed within a cavity. The distance between the magnet and the container is adjustable so as to create a desired magnetic field strength within the magnetic field cavity of the test medium. The apparatus may include means for adjusting the distance between a magnet and the container.

The magnetic field strength in the cavity is normally stronger at a part of the internal surface of the container closer to the magnet (locus of magnetic force) than it is elsewhere in the cavity and becomes negligible outside the cavity. As a result, magnetic particles near this locus are subject to considerably greater magnetic force than those farther from it. In certain preferred embodiments, two magnets may be located on the opposite sides of the container, preferably with similar magnetic poles facing each other, to distort the magnetic flux lines and generate two magnetic field gradients and two loci of magnetic force forming in one cavity. Such an arrangement is particularly useful for agitating magnetic particles, as described below. In a particularly advantageous arrangement, an assembly comprising a vertical array of magnets are positioned exterior to the container to create multiple magnetic field gradient cavities within desired cross-sections of the test medium.

The present invention provides for agitating and mixing the magnetic particles within the test medium while maintaining the test medium substantially motionless with respect to the container. The magnetic particles are moved through the test medium by rotating the container with respect to a stationary magnet defining a stationary magnetic field gradient cavity. This motion induces an angular movement in the magnetic particles relative to the substantially

motionless test medium caused by the change in angular position between the aggregated particles within the container and the magnet. The magnetic particles are also moved within the test medium by moving a magnet defining a moving magnetic field gradient cavity along a container. This movement induces an angular movement of the particles relative to the substantially motionless test medium caused by the change in angular position between the magnet and the aggregated particles.

A motionless magnetic field gradient cavity with respect to particles tends to aggregate the magnetic particles in the test medium as a relatively compact mass on the inner surface of the lateral wall of the container closest to magnetic means. As the particles are all clustered in the vicinity of the magnetic means, they also tend to stick to each other by non-magnetic forces of compression and surface tension. The degree of compression naturally depends on the force of magnetic attraction and is particularly relevant in the case of particles with diameters of a few microns, such as are usually employed in affinity separation. Such compacted particles can remain aggregated even after the removal of the magnetic field and usually require vigorous shaking of the test medium to re-disperse. A carefully balanced magnetic field strength in the test medium will pull the particles out of suspension into an aggregate, but will not be so strong as to overly compress the aggregate.

This is particularly important in the present invention with respect to the mixing operation. As the relative angular position between the container and the magnet is displaced at a sufficiently rapid rate, the aggregated mass of particles move with the wall of the container to a position of weaker magnetic field. At this position, the stronger magnetic field in the vicinity of the magnetic means begins to pull off the particles from the aggregated mass, the trajectories of the particles being, pulled off depending on the angular position of the aggregated mass and magnet. As the particles are pulled, they move and form chains of particles, due to the induced magnetic dipole on the particles by the applied magnetic field. As the chains accelerate towards the magnet, fluid drag forces cause them to break creating a cloud of magnetic particles in the fluid medium. During continuous rotation, the relative angular position between the magnet and the internal surface of the container bearing the aggregated particles recedes continuously and causes the particles to move ceaselessly in angular trajectories within the test medium thereby enabling the re-suspension and mixing of magnetic particles.

The displacement of particle trajectories in a continuous manner is based on the action of magnetomotive force acting at a continuously changing angle between the magnet and the

paramagnetic particles which results in a mixing process without fluid turbulence. Furthermore, this mixing process significantly increases the collision frequency between the particles and target species thereby enhancing the efficiency of the affinity binding reaction.

The break-up of particle chains as described above may be aided by providing additional means to abruptly change the polarity of the magnet. For example, if the north pole of the magnet is facing the container, it may be flipped to the south pole. The repulsive forces generated by such sudden reversal of a magnet pole aids in the breakup of a particle chain. Such magnet pole flipping may be accomplished by any rotation device. The frequency of flipping may vary is desired. In general, a specific rate of change in the angular position of the container and magnet, i.e., speed of rotation, to ensure re-suspension and mixing to a large extent depend on the size, density and magnetic susceptibility of the particles, the cross sectional diameter of the container, the density and viscosity of the fluid test medium and the strength of the magnetic field. As regards particles it should be noted that the force pulling a magnetic particle through a fluid medium is the product of its magnetic saturation and field gradient and the viscous force opposing particle motion, which is governed by Stokes Law. A suitable speed of rotation can be calculated on the basis of forces of gravity, buoyancy, fluid friction and magnetism. However, for a given set of parameters, the intensity of the magnetic field or fields and the appropriate speed of rotation will be modulated experimentally. It should be noted that too high a rotation speed will not allow the particles sufficient time to detach from the aggregated mass and particles will be spread over the circumference of the inner wall of the container. Similarly, too slow a rotation speed will produce a rolling mass of the aggregated particles. In both cases, re-suspension and mixing of the particles will be prevented. The field strength in the magnetic field cavity of the test medium must also be balanced so as to allow the aggregated particles to move with the wall of the container. It will be appreciated that a fixed magnet position is inconvenient when the desired particle size may vary considerably. In such situations, it is advantageous to be able to adjust the distance between the magnet and the container to create the optimum field, strength in the magnetic field cavity of the fluid medium.

Although a continuous rotation in the sense described above usually provides satisfactory mixing of magnetic particles, in certain situations it is advantageous to provide a step-wise change of a predetermined distance in angular position. For example, the relative angular position may be changed to 90 or 180 degrees in a single step. Such steps may be repeated more than once. If desired, time delays may be imposed between such steps.

The separation of magnetic particles from the liquid test medium in accordance with the invention is effected by stopping the rotation of the magnet with respect to the container to terminate the agitation of the magnetic particles. In the stationary position between magnet and aggregated particles, the magnetic particles within the magnetic field gradient in the fluid medium are attracted to and immobilized at the inside wall of the container nearest to the magnet.

The need for a reliable and readily automated method for re-suspending and mixing the aggregated magnetic particles without causing fluid turbulence has not been satisfactorily addressed. Applicant's invention utilizes a new principle of which has allowed, for the first time, integration of a simplified mixing and separation process into a single device.

The present invention provides many advantages over the prior art devices for affinity magnetic separation. The mixing of the present invention provides a high rate of contact between the affinity surface of the magnetic particles and the target substance, thereby enhancing the affinity bonding, without causing fluid turbulence. As a consequence, the hydrodynamic shear forces remain low and will not affect the affinity bond between particle and target substance complex or prevent denaturation, or other damage to the target substance. The process of the present invention can be used for sample volumes as little as 100 μL and can be scaled up to process sample volumes in excess of 100mL. The present invention is particularly useful for the isolation of human rare cells required in various cell therapies as it permits a level of operating efficiency which has not been achievable before this.

The purity and yield of the target substance obtained by a particular affinity magnetic separation is largely determined by the mixing process employed to promote the affinity binding reaction between the target substance and the surface of the magnetic particles. The binding reactions require a close contact between the affinity surface and the target substance. The rate of the reaction largely depends on the collision frequency between the two entities and the rate of surface renewal of the magnetic particles. The surface renewal is the process of removing the thin layer of media at the affinity surface and exchanging it with fresh media from the bulk. The hydrodynamic shear force at the affinity surface, therefore, must be carefully balanced so that it is sufficient to remove the thin layer of media without disrupting the affinity bonds. This has been difficult to achieve by past mixing methods based on agitating the fluid medium. The present invention, however, provides a high collision frequency and a

substantially balanced shear force by magnetically inducing a controlled movement of the magnetic particles in an essentially motionless fluid medium.

In affinity magnetic separation, the particle concentration is, typically, much greater than the target substance to enhance the yield of the target substance. This is particularly important in the isolation of rare cell types such as mammalian hemopoietic cells where a particle to cell ratio of 20:1 may be required to obtain a desired isolation efficiency. In such applications, magnetic beads of uniform size distribution are required. The high cost of these beads are widely appreciated. The ability to isolate highly purified stem cells may serve in the treatment of lymphomas and leukemias as well as other neoplastic conditions. However, for the isolation of human stem cells, processing of large sample volumes is required. Such a process consumes large quantities of magnetic beads. Thus there is a need to reduce the concentration of magnetic beads without sacrificing the required high purity and yield. One embodiment of the present invention is capable of treating large sample volumes by relatively small concentrations of paramagnetic particles by combining a vertically moving magnet along the length of the container while the container is rotating.

The mixing and separation process of the present invention have particular utility in various laboratory and clinical procedures involving biospecific affinity binding reactions for separations. In such procedures, magnetic particles are used which have their surface coated with one member of a specific affinity binding pair, i.e. ligand or receptor, capable of specifically binding a substance of interest in the test medium.

Such biospecific affinity binding reactions may be employed for the determination or isolation of a wide range of target substances in biological samples. Examples of target substances are, cells, cell components, cell subpopulations (both eukaryotic and prokaryotic), bacteria, viruses, parasites, antigens, specific antibodies, nucleic acid sequences and the like. The apparatus and method of the invention may be used to carry out immunospecific cell separations for the analysis or isolation of cells including, by way of example: tumor cells from bone marrow; T-lymphocytes from peripheral blood or bone marrow; lymphocyte subsets, such as CD2, CD4, CD8, and CD34 from peripheral blood, monocytes; granulocytes and other cell types. The removal or depletion of various cell types may be carried out in a similar manner. The invention may be also be used in the separation or, analysis of various bacteria or parasites from food products, culture media, body fluids and the like. Similarly, the apparatus and method of the present invention may be used in: bioassays including immunoassays and nucleic

acid probe assays; isolation and detection of DNA and mRNA directly from crude cell lysate; and isolation and detection of proteins.

The magnetic particles preferred for the practice of the invention are noncolloidal paramagnetic or superparamagnetic particles. Such magnetic particles are typically of polymeric material containing a small amount of ferro-magnetic substance such as iron-based oxides, e.g., magnetite, transition metals, or rare earth elements, which causes them to be captured by a magnetic field. The paramagnetic particles useful for practicing the invention should provide for an adequate binding surface capacity for the adsorption or covalent coupling of one member of a specific affinity binding pair, i.e. ligand or receptor. The preferred diameter of a particle is typically in the range between 0.1 to 300 μm. Suitable paramagnetic particles are commercially available from Dynal Inc. of Lake Success, NY; PerSeptive Diagnostics, Inc., of Cambridge, MA; and Cortex Biochem Inc., of San Leandro, CA. The preferred particles are of uniform size between about 1 and 5 μm in diameter, and contain magnetizable material evenly dispersed throughout. Such particles may be obtained from Dynal under the identification numbers M-280 and M- 450 by Dynal Inc. These beads are coated with a thin shell of polystyrene which provides a defined surface for the immobilization of various ligands or receptors. Such immobilization may be carried out by any one of many well-known techniques; techniques employing either physical adsorption or covalent coupling chemistry are preferred.

The magnetic field gradients may be generated by one or more permanent magnet(s) or electromagnet(s). Permanent magnets are generally preferred for use in laboratory-scale operations and for automated devices employed in clinical diagnostics are preferred. However, larger scale devices or automated devices such as those employed in pharmaceutical or industrial production can be more advantageously produced using electromagnets, since the field gradients can be more easily altered under automatic control to effect various processing steps.

Permanent magnets for practicing the invention preferably have a surface field strength sufficient to attract a majority of the magnetic particles. Permanent magnets of rare earth alloys having a surface field strength in the range of several hundred Gauss to several kilo-Gauss are preferred. High energy permanent magnets made from Neodymium-Iron-Boron or Samarium-Cobalt magnets and characterized by BHmax (maximum energy product) in the range of 25 to 45 MGOe (megaGauss Oersted) are particularly preferred. Such magnets may be obtained from

International Magnaproducts Inc., of Valparaiso, IN, and many other commercial sources. Preferably the permanent magnets have a rectangular cross-section and may be glued or fixed by mechanical means to a nonmagnetic holding support to form a permanent magnet assembly. The assembly may include a ferromagnetic harness to house the magnet or magnets and to intensify and focus the magnetic field. The magnets are preferably oriented with their magnetic lines of force perpendicular to the vertical axis of the container. Alternate cross-sectional shapes, orientations, and magnetic pole orientation with respect to the container are also envisioned.

Generally the permanent magnet assembly is placed in close proximity to the container without the magnet extending to the bottom of the container. The distance between each magnet and the container shown in Figures 2 and 3 is adjustable between about 1 mm to about 20 mm to create a desired magnetic field strength within the magnetic field cavity of the test medium. The apparatus shown in these figures includes a means for adjusting the distance between each magnet assembly and the container. Depending on the size and magnetic susceptibility of the particles and the field strength of the magnets and cross-section diameter of the container, the appropriate distance will be determined experimentally. The field strength created in the magnet field cavities should be carefully balanced so that it is sufficient to pull the particles out of suspension, aggregate the particles on the side of the container, and allow the aggregated particles to move with the wall of the container. However, the magnet may be moved closer to the container, as discussed, to increase the field strength in order to separate the particles from the liquid test medium. In certain situations involving the processing of a plurality of containers, it may be advantageous to place the permanent magnet assembly between containers or between rows of containers so that one single permanent magnet assembly can be used to generate a magnetic field cavity in the two containers in its vicinity.

Figure 3 illustrates a preferred embodiment of the present invention for processing a plurality of test liquid media simultaneously. It includes a linear drive mechanism mounted on a positioning mechanism and a rotation mechanism. The three mechanisms allow vertical linear movement of a magnet assembly, adjustment of the distance between the magnet assembly and containers, and rotation of the containers. Simultaneous container rotation and linear magnet movement provides the advantage of processing large volumes of test media with a relatively small quantity of magnetic particles.

The apparatus of Figure 3 consists of two main parts, linear drive assembly 111 and base assembly 112. Both assemblies are constructed of a nonmagnetic material, aluminum being preferred. The linear drive assembly 111 comprises a rigid frame 113 with two fixed guide rods 114 and 115 and a centrally located screw shaft 116. The end portions of screw 116 are smooth and un-threaded and are mounted in two centrally located ends flanges (not shown). The screw 116 is freely rotatable and includes a roll nut (not shown) which moves linearly in the vertical plane, either up or down, upon rotation of screw 116. A pulley 117 is fixed to the smooth portion of screw 116 protruding from the top plate 138 of frame 113 and is connected by a timing belt 118 to another pulley 119 fixed to the shaft of a variable speed electric motor 120 mounted on bracket 121 of frame 113. Timing belt 118 is made of neoprene or urethane with precisely formed grooves on the inner side. The belt width and groove pitch match the dimensions of the teeth on pulleys 117 and 119 to provide positive and non-slip power transmission. Suitable timing belts and gear pulleys may be obtained from Stock Drive Products, New Hyde Park, NY or from other similar vendors.

A carriage 122 is fixed on the roll nut (not shown) of screw 116. Its vertical motion is ensured by the accurately aligned guide rods 115 and 114. Linear drive assembly 111 is attached to base assembly 112 by bolting the bottom plate 139 of frame 113 to a linear slide mechanism 123. A rod with a knob 128 inserted through a center hole of the base assembly 112 is attached to the linear slide mechanism 123. The linear slide mechanism 123 thus can be moved forward or backward by pulling or pushing the knob 128 to position it at a desired distance from the containers 124.

A magnet assembly 125 with magnets 126 is removably mounted on the linear drive carriage 122 by means of three evenly spaced screws 127. This is advantageous because magnets of varying size and geometry can be easily exchanged. The magnets 126 are aligned with the row of containers 124. Their distance from the containers is adjusted by pulling or pushing the knob 128.

The motor 120 rotates the screw 116. The roll nut (not shown) converts this rotary motion to a linear motion moving magnet assembly 125 vertically. The direction of the linear movement of magnet assembly 125 is controlled by the clockwise or counter-clockwise rotation of the motor 120 by a motor controller (not shown). The movement of magnet assembly 125, either upward or downward can thus be controlled at will and may be repeated for as many cycles as desired.

The position and the stroke length of the linear up and down movement of the carriage 122 may be controlled by two position sensors (not shown) to control the lowest and highest extremes of travel of the carriage 125. An electronic signal from these sensors may be used to reverse the motor rotation, thereby causing a repeated scanning for a desired length of the containers 124 by their corresponding magnets 126.

Electronic motor controllers and position sensor are well known in the art and may be obtained from any one of a number of vendors. If permanent magnets are employed, they are preferably a rare earth, type as described above and have suitable dimensions and geometries so as to define a magnetic field cavity of a desired field strength which provides a desired cross-section within the liquid test medium in each container.

The base assembly 112 includes a mechanism for rotation comprising a variable speed electric motor 129 with a gear pulley 130 fixed to its shaft. A pulley rotor 131 is attached to each one of a plurality of holder 134. A timing belt 132 is wrapped around the gear teeth of pulley 130 and each of the rotors 131. Although only one rotor 131 is shown next to a holder 134 for a container 124, it should be understood that each container holder 134 has a rotor 131 associated with it which is driven by the belt 132. The motor 129 and rotor pulleys 130, 131 are secured in their precise positions by a top metal plate 133 fixed to base assembly 112. It should be noted that the gear pulley rotors 131 are free rotating and their respective shafts protrude from corresponding holes in plate 133. The belt width and the inner groove pitch of the timing belt 132 dimensionally match with gear teeth of the motor gear pulley 130 and the rotors 131 to provide positive and non-slip power transmission. If desired, idling rollers may be installed between the pulleys to increase the wrap around the gear teeth for a firmer non-slip power transmission. The motor 129 rotates the timing belt 132 thereby simultaneously rotating all pulley rotors 131.

Holders 134 are removably mounted on the tapered end of a rotor shaft 135 protruding from corresponding holes in plate 133 and provide means for funny holding containers 124 in a substantially vertical position. A removable holder design is advantageous as it provides a convenient means to accommodate a variety of container sizes on the apparatus by simply changing the holders to correspond to the container geometry.

The position of the magnet assembly 125 may be adjusted to a required distance from the row of containers 124. The motor 129 rotates containers 124 around their vertical axes. As

containers 124 rotate, the relative angular position of the aggregating magnetic particles in each container with respect to its corresponding magnet 126 is continuously altered, inducing the magnetic particles to mix within the cavity of the magnetic field gradient, as described above. While the containers 124 are rotating, motor 120 may be switched on to move the magnets 126 up and down in the vertical plane thereby moving the magnetic field cavity in alignment with the vertical axis of the containers. Upon reaching a desired length of the container, the direction of movement of magnet assembly 125 is reversed. This process is repeated for the entire duration of particle mixing.

It will be recalled that the magnetic particles remain confined in the magnetic field cavity. Particle to target substance ratio therefore may be adjusted to relatively high levels within the magnetic field cavity to provide reaction conditions which overwhelmingly favor affinity binding. By combining a linearly moving magnetic field cavity with the angular movement of particles confined within the magnetic field cavity, a simple and efficient means to process large volumes of test media without a concomitant increase in particle concentration is obtained. This was not heretofore possible.

The motor 129 may be an electric step motor to provide a step-wise change of a predetermined distance in the relative angular position such as described above. Similarly, motor 120 may be an electric step motor to provide a step-wise change of a predetermined distance in the vertical plane. Various combinations of continuous and step-movement for the rotation and linear movement maybe utilized. In every case the optimum speed of rotation and linear movement will be determined by trial and error.

For separation, the linear drive motor 120 is turned off. The magnet assembly 125 is brought to a home position. The rotation drive motor 129 is turned off. The magnetic particles in the containers 124 are attracted to and immobilized at the inside wall closest to the magnets 126. The aggregation of the magnetic particles on the vertical side of the container 124 facilitates removal of the test medium by aspiration or similar methods. If desired, magnet assembly 125 may be moved closer to containers 124 by moving knob 128. This tightly aggregates the magnetic particles on the walls of the containers 124 to facilitates a clean removal of the test medium.

Figures 1A through 1f illustrate the preferred steps in a method practiced by the preferred embodiments described above, using affinity reactive magnetic particles of about

2.8 μm for the purpose of bioassays, or for the isolation of cellular or molecular species from a sample solution or suspension of biological fluids.

Figure 1A shows an apparatus in which a suspension of magnetic particles 58 in a sample solution is dispensed with a pipette 59 into a test tube 23 of about 10 mm diameter. A magnet 21 with a surface field of about 400 Gauss, is moved to a distance of about 5 mm from test tube 23. This preferred distance was determined by experiment. The motor is turned on and the magnetic particles 58 are mixed by rotating the magnet 21 around the test tube 23. Figure 5b shows the same apparatus when mixing is completed, rotation of the magnet 21 has stopped, and the magnet is moved closer to the test tube 23. The magnetic particles 58 are immobilized against the inner wall of test tube 23 closest to the stationary magnet 21.

Figure 1C shows the apparatus during a washing step. In this step, an outlet tube 59a aspirates the supernatant test medium and an inlet tube 59b adds a suitable wash solution into the test tube 23. The magnetic particles 58 are then mixed in the wash solution. The old wash solution is aspirated and new clean solution may be added. The washing step may be repeated as many times as required.

Figure 1D shows the apparatus stopped for the addition of one or more reagent solutions by pipette 59 for effecting a desired analytical reaction for a bioassay a chemical displacement reaction to elute the target substance from the magnetic or particles 58.

Figure 1E shows the same apparatus turned on for dispersing and mixing the magnetic particles 58 for carrying out the desired reaction.

Figure 1F shows the apparatus stopped to separate the magnetic particles 58 from the reaction medium. In the case of bioassays, the supernatant liquid may be measured by any desired measurement method, either directly in test tube 23 or by transferring it elsewhere. For the purpose of isolating a cellular or molecular species, the supernatant may be transferred to a suitable container for subsequent treatment as desired. Examples of actual separations of mRNA and protein are described in a technical brochure entitled "MixSep," obtainable from Sigris Research, Inc., and is incorporated herein in its entirety.

As mentioned above, permanent magnets and electromagnets are interchangeable in most configurations of the present invention. However, those configurations that require movement of a magnet are more easily realized with permanent magnets. Electromagnets

require commutators or other arrangements to conduct electricity to the moving magnets. There are certain unique configurations in which electromagnets are greatly preferred. Figure 2A shows two electromagnet coils 101a and 101b mounted on a support frame 104 and displaced at about 180 degrees at the exterior of a container 102 with the liquid test medium and magnetic particles 103 inside. Figure 2B shows a cross-section of a single container 102 with the liquid test medium and magnetic particles 103 surrounded by a ring of individual electromagnet coils 101a to 101r mounted on a support frame 104.

Here neither the container 102 nor the electromagnets 101 actually move. Instead, angular movement is induced in the magnetic particles suspended within the test medium 103 inside the container 102 by sequentially energizing the electromagnets. This sequential energization may be "binary" (i.e., on and off) or "analog," in which a first electromagnet is gradually fully energized, and then has its power reduced, while the next electromagnet is gradually energized, and so on. It will be apparent that rate of motion of the magnetic particles 103 can be modulated by the rate of change and the degree of overlap between the sequential electromagnets.

The exact number of sequential electromagnets employed will depend on the size of the container 102 and other parameters. The angular movement from one magnet to the other in its simplest form is 180 degrees so that the magnetic particles in the test medium 103 will move in relatively straight lines back and forth across the container 102. More variety is preferably added to the paths of the magnetic particles by modulating the polarity, as well as the power level of the electric current, thereby altering the direction of the magnetic poles with alterations of the magnetic field.

It has been found that a configuration employing four electromagnets equally spaced (i.e., 90 degrees apart) around a container can produce very acceptable agitation of magnetic particles through a judicious use of sequential activation of the electromagnets and through polarity reversals, as discussed above.

The container defining the mixing and separation chamber includes at least one opening for the addition and removal of a test medium. The container is preferably of substantially cylindrical form and made from a magnetically permeable material such as plastic or glass. Additionally, the inside surface of the chamber may be biocompatible and, if desired, the chamber may be sterilized for aseptic processing of the test media. The volume of the container

is not critical as long as an adequate magnetic field gradient can be provided to accommodate the chamber and, particularly, can accommodate the desired cross-section of the liquid test medium inside.

The container used to hold the test medium may be a test tube or an eppendorf type of tube with a conical bottom. The volumetric capacity of the test tube is preferably between 250 μl to about 18 ml as usually employed in research laboratories. The various configurations of apparatus as described above can be easily scaled up to process much larger volumes of liquid test media as may be required for clinical applications. In all cases, the size and geometry of the magnet is adjusted to generate an adequate magnetic field strength within the field cavity of the test medium inside a particular size of container.

Although embodiments of the present invention particularly suited for use in the research laboratory preferably employ readily removable and replaceable containers such as test tubes, diagnostic and other devices employing the teachings of the present invention might employ permanent flow cells or other non-removable chambers for mixing and separation.

Those skilled in the art will appreciate that various adaptations and modifications of the just-described preferred embodiment can be configured without departing from the scope and spirit of the invention wherein the affinity reactive magnetic particles are admixed with the liquid test medium in a container by effecting a relative angular movement of the magnetic particles in the liquid test medium, while the liquid remains essentially motionless. The relative angular movement is induced in the magnetic particles by either rotating a magnetic field around a stationary container or rotating the container relative to an immobile magnetic field. The magnet creating the field is disposed outside the container and defines a cavity of magnetic field gradient within the liquid test medium. Any container configuration may be utilized, such as, for example, a doughnut-shaped container. In such a container the magnetic source may be "outside" of the container and "within" the container, if it occupies the hole of the doughnut. Therefore, it is to be understood that, within the scope of the appended claims, the invention may be practiced other-wise than as specifically described herein.

CLAIMS

What Is Claimed Is:

1. An apparatus for mixing magnetic particles in, and separating magnetic particles from a liquid medium, the apparatus comprising:

a magnetically permeable container 124 having walls containing a liquid medium;

a quantity of magnetic particles 58 located in the liquid medium in said container;

a magnet 126 disposed outside the walls of said container generating a magnetic field gradient inside the container in a portion of the liquid medium, defining a magnetic field cavity in the liquid medium;

means 129 for continuously changing the relative angular position between said magnetic particles in said container and said magnet causing movement of said magnetic particles throughout the magnetic field cavity in the liquid medium; and

means 121, 116 for moving said magnet concurrently with said continuously angularly changing means along the outside walls of said container from one end of the liquid medium to the other, causing the magnetic field cavity to move from one end of the liquid medium in said container to the other, while said magnetic particles are moving throughout the magnetic field cavity.

2. The mixing and separating apparatus of Claim 1 wherein said magnetic particles may range in diameter from about 0.1 μm to about 300 μm.

3. The mixing and separating apparatus of Claim 1 wherein said magnet may range in strength from about 200 Gauss to about 5000 Gauss.

4. The mixing and separating apparatus of Claim 1 further comprising means for moving said magnet closer to or further away from the walls of said container.

5. The mixing and separating apparatus of Claim 1 wherein said means for continuously changing the relative angular position, continuously rotates said container on a concentric axis relative to a stationary magnet at between 10 to 200 revolutions/minute.

6. The mixing and separating apparatus of Claim 1 wherein said means for moving said magnet from one end of the liquid medium to the other continuously moves said magnet.

7. The mixing and separating apparatus of Claim 1 wherein said container is step-rotated on its concentric axis.

8. The mixing and separating apparatus of Claim 1 wherein the step rotating movement of the container includes a predetermined time delay between the step increments.

9. The mixing and separating apparatus of Claim 1 wherein said means for moving said magnet from one end of the liquid medium to the other moves said magnet in step increments.

10. The mixing and separating apparatus of Claim 9 wherein the step increment movement of said magnet includes a predetermined time delay between the step increments.

11. The mixing and separating apparatus of Claim 1 wherein said container comprises a plurality of containers; a quantity of magnetic particles located in each one of said containers; a magnet disposed outside each one of said plurality of containers; each container having a relative angular position changing means; and each one of said magnets having a means for moving the magnet from one end of the liquid medium to the other.

12. An improved method of mixing affinity reactive magnetic particles and a liquid test medium in a container causing an affinity binding reaction between a target substance in the liquid test medium and said magnetic particles, said method maximizing contact between the affinity surface of the magnetic particles and the target substance without liquid turbulence, by keeping said liquid medium substantially motionless with respect to said container, the method comprising the steps of:

placing the liquid test medium and the magnetic particles into a magnetically permeable container having walls;

generating a magnetic field gradient inside said container in a portion of the liquid test medium to define a magnetic field cavity within the liquid medium by a magnet disposed outside of the walls along a portion of the container;

adjusting the field strength in the said magnetic field cavity by adjusting the distance between the magnet and the container;

changing the relative angular position between the magnetic particles aggregating on the walls of the container and the magnet to mix the magnetic particles throughout the magnetic cavity in the liquid test medium; and

13. concurrently with said angularly changing step, moving the magnet along a vertical length of the container for a distance along the liquid test medium to scan a desired length of the liquid test medium with the magnetic field cavity containing the agitating magnetic particles.

14. The method of Claim 12 wherein said step of moving the magnet along the length of the container is repeated as desired.

15. The method of Claim 13 wherein said step of moving the magnet along the length of the container step further includes varying the length of movement along the liquid test medium.

16. The method of Claim 12 further comprising the steps of -

stopping movement of the magnet at a desired position along the length of the container;

keeping constant the relative angular position of the magnet and the magnetic particles in the container concentrating the magnetic particles from the liquid test medium on an inside surface of the container;

moving the magnet closer to the walls of said container to firmly aggregate the concentrated magnetic particles; and

17. removing the liquid from the container without disturbing said concentrated particles.

18. The method of Claim 12 wherein said step of changing the relative angular position between the magnetic particles and the magnet comprises rotating the container on a concentric axis relative to a stationary magnet at between 10 to 200 revolutions/minute.

19. The method of Claim 16 wherein said rotating step comprises rotating the container in step increments of a preselected angular distance at predetermined time delays between step increments.

20. The method of Claim 12 wherein said generating step comprises a plurality of electromagnets spaced apart around the perimeter of the container outside the walls; and wherein said step of changing the relative angular position between the magnetic particles and the magnet comprises sequentially energizing the plurality of magnets sequentially around the container.

21. The method of Claim 12 further comprises the step of generating at least one additional magnetic field gradient inside the container in another portion of the liquid medium to define at least two separate magnetic field cavities in the liquid medium.

22. The method of Claim 12 wherein said magnet is moved along a vertical length continuously.

23. The method of Claim 12 wherein said magnet is moved along a vertical length in step increments at predetermined time delays between step increments.

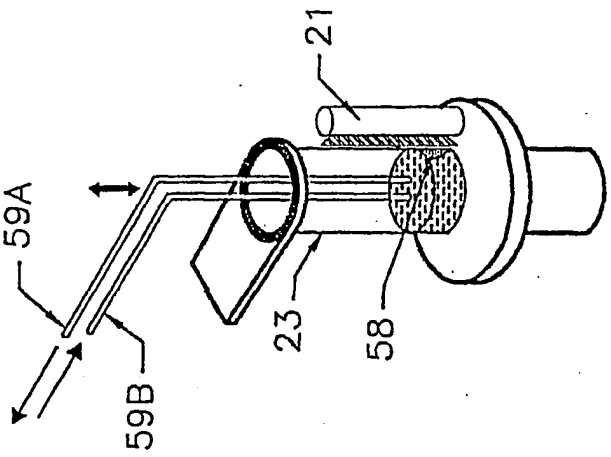

FIG. 1C

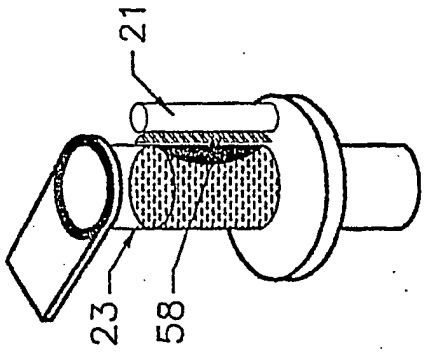

FIG. 1B

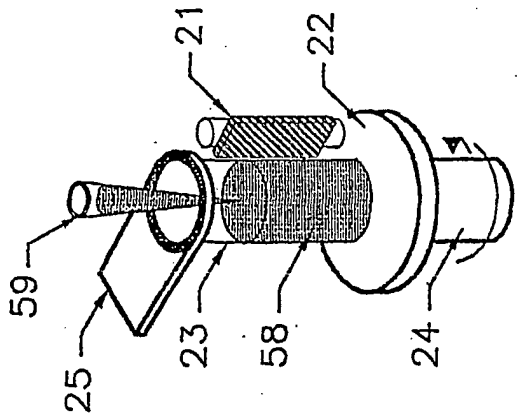

FIG. 1A

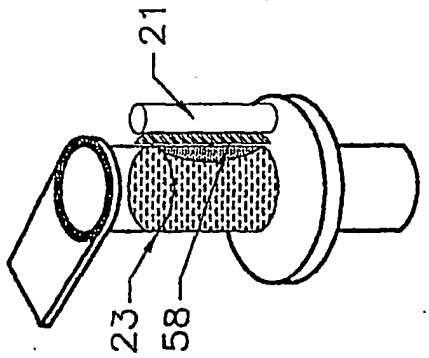

FIG. 1F

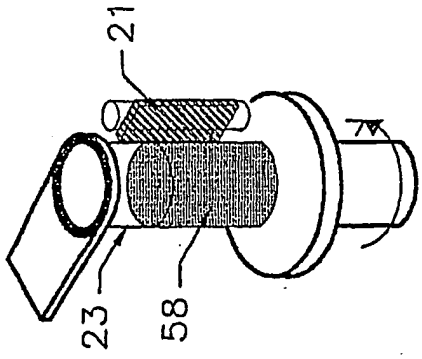

FIG. 1E

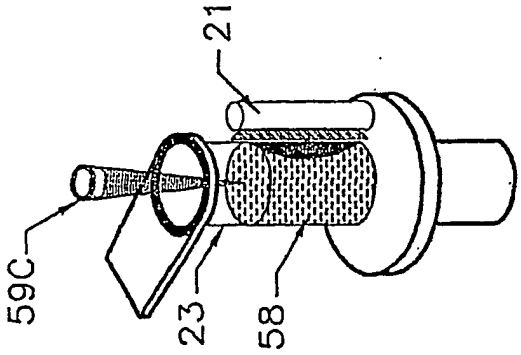

FIG. 1D

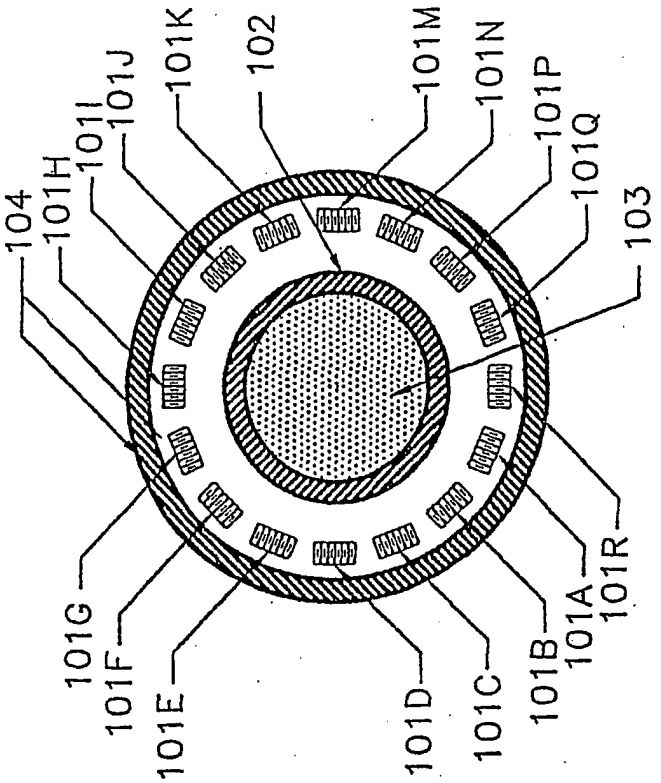
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FIG. 2B

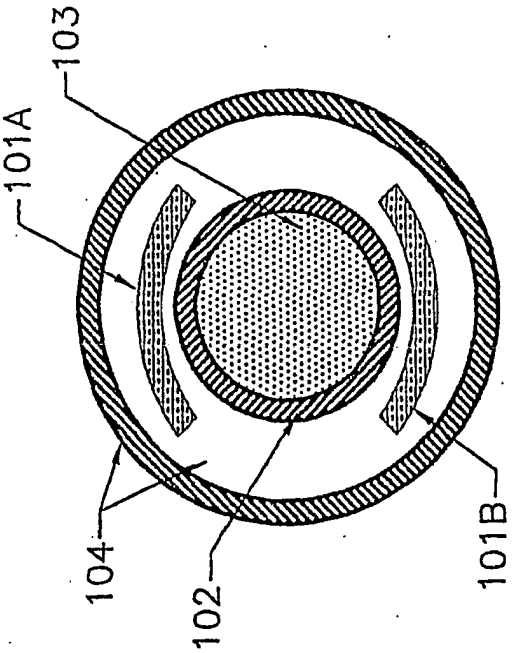
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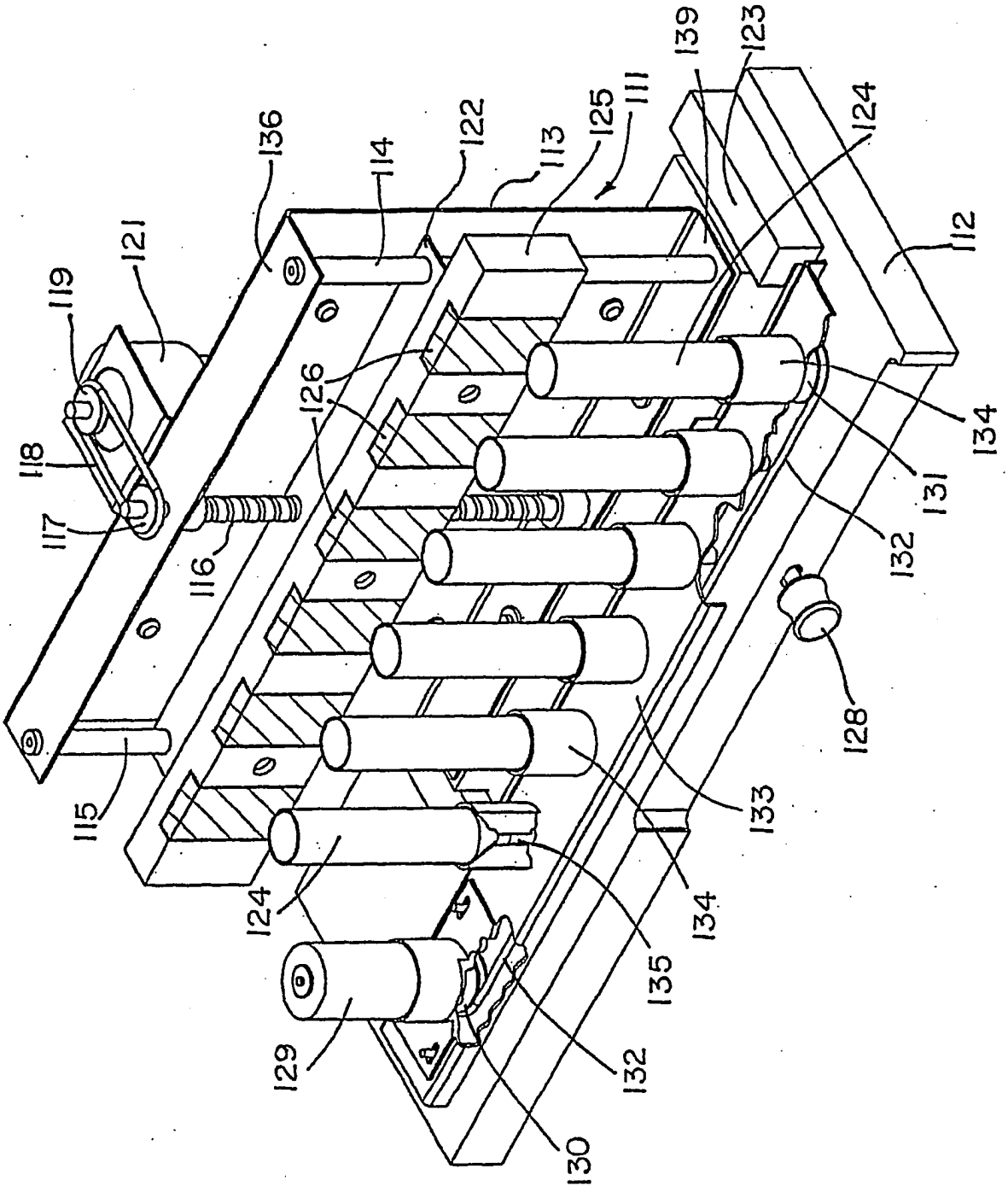
FIG. 2A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INTERNATIONAL SEARCH REPORT

Inte nal Application No
PCT/US 00/00071

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 B03C1/28 B03C1/01 G01N33/543

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 B03C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
PAJ, EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PATENT ABSTRACTS OF JAPAN vol. 006, no. 127 (C-113), 13 July 1982 (1982-07-13) & JP 57 053257 A (TOHOKU METAL IND LTD;OTHERS: 01), 30 March 1982 (1982-03-30)	1
A	abstract	12,20
Y	WO 96 26011 A (SIDDIQI IQBAL W) 29 August 1996 (1996-08-29)	1,2
A	page 13, line 17 - line 20	3,5,11, 18,20
	page 14, line 6 - line 29	
X	page 17, line 21 -page 18, line 26; claims 1,12; figures 1,4,10	12
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 August 2000	30/08/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Decanniere, L

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INTERNATIONAL SEARCH REPORT

Inte ›nal Application No
PCT/US 00/00071

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
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A	PATENT ABSTRACTS OF JAPAN vol. 008, no. 030 (C-209), 8 February 1984 (1984-02-08) & JP 58 193687 A (SHINGIJIYUTSU KAIHATSU JIGIYOUDAN;OTHERS: 01), 11 November 1983 (1983-11-11) abstract	12,20
E	US 6 033 574 A (SIDDIQI IQBAL W) 7 March 2000 (2000-03-07) column 15, line 31 -column 17, line 39; claims 1-10; figure 12	1-23

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